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Tetraploid-octoploid relationships and karyological evolution in the order Acipenseriformes (Pisces)
Karyotypes, nucleoli, and nucleolus-organizer regions in four acipenserid species

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Abstract

The karyotypes of four Acipenseriformes species, *Acipenser gueldenstaedti*, 2n = 250±8, *A. ruthenus*, *A. stellatus* and *Huso huso*, 2n = 118±2, are described. In all four karyotypes the majority of chromosomes are meta- and submetacentric macrochromosomes, and microchromosomes of different morphology make up about one third of the set. In *A. ruthenus* the NORs are located in the telomeric region of a pair of microchromosomes and at least in one pair of middle-size acrocentrics, and in *A. stellatus* and *Huso huso* also in the telomeric regions of at least one pair of microchromosomes. The modal number of active nucleoli in *A. gueldenstaedti* nuclei amounts to 6–8 (range 2–12), in *A. ruthenus*, *A. stellatus* and *H. huso* nuclei to 2–3 (range 1–6). The data obtained point to the tetraploid origin of Acipenseriformes species with 120 chromosomes and to the octoploid origin of species with 240–260 chromosomes.

Introduction

Fishes of the order Acipenseriformes represent an evolutionary ancient group, karyologically not sufficiently known yet. Results of some investigations show very unusual features of their karyotypes, namely they consist of a great number of chromosomes, about half of them being microchromosomes (Fontana & Colombo, 1974; Fontana *et al.*, 1977; Dingercus & Howell, 1976; Vasiliev *et al.*, 1980; Vasiliev, 1985; Arefiev, 1983). Moreover, this order is evidently characterized by polyploidy since it includes species having 120 and 240 chromosomes. It was proposed that the 120-chromosome forms are tetraploids, while their hypothetic extinct ancestors had karyotypes consisting of 60 chromosomes (Dingercus & Howell, 1976; Vasiliev *et al.*, 1980). This work describes the karyotypes of four Acipenseridae species which inhabit the European part of the USSR, the localization of NORs and the number of active nucleoli per nucleus of each species. The data indicate the tetraploid origin of sterlet, sevruga, beluga, and the octoploid origin of Russian sturgeon.

Material and methods

The young of the current year of Russian sturgeon (*Acipenser gueldenstaedti*), sterlet (*A. ruthenus*), sevruga (*A. stellatus*) and beluga (*Huso huso*) were used; the young were obtained by artificial breeding at the Ikryaninsky fish-breeding factory (Volga river). After intramuscular injection of 0.3–0.5% colchicine solution the fishes were kept for 7–8 h in well aerated water. After that the lymphoid organs of their heads were prepared and placed into hypotonic solution (0.9% sodium citrate) for 30 min. Then they were fixed in cold alcohol-acetic acid mixture (3:1). For slide preparation a piece of the fixed lymphoid organ was suspended in a few drops of the fixative mixture, placed onto the surface of a glass slide and the suspension was air dried. The slides were stained with Giemsa solution or with ammoniacal silver according to the Goodpasture and Bloom (1975) method. Our detailed descriptions of the chromosome analysis of Acipenseriformes and of Ag-AS-staining were published earlier (Vasiliev & Sokolov, 1980; Birstein, 1981).

Results

The karyotypes

Results of the chromosome analysis of the four species investigated are listed in Table 1, and karyograms are shown in Figures 1–4. The karyotypes of three of the four species consist of 118 ± 2 chromosomes, and the *Acipenser gueldenstaedti* karyotype of 250 ± 8 chromosomes. As the microchromosomes are very small and make up about one third of the whole set, it was impossible to count the exact chromosome number. Moreover, each of the species may be polymorphic in microchromosome number. The morphology of many microchromosomes is unclear because of their very small size. All these difficulties result in approximate numbers of meta- and submetacentric chromosomes in Table 1.

Nucleoli and nucleolus organizers

The mean number of active Ag-staining nucleoli per nucleus in *A. gueldenstaedti* is considerably greater than in the other three species (Table 2; Figs. 5a, b). In *A. gueldenstaedti* there are no nuclei with one nucleolus, the number of nuclei with 2–3 and 12–12 nucleoli is small (7.5%), nuclei with 6–8 nucleoli are the most frequent, and the modal class is 7 nucleoli. The distribution of nuclei with different numbers of nucleoli in three other species is almost the same: nuclei with only one stained nucleolus form 9.5–15.0% (depending on the spe-

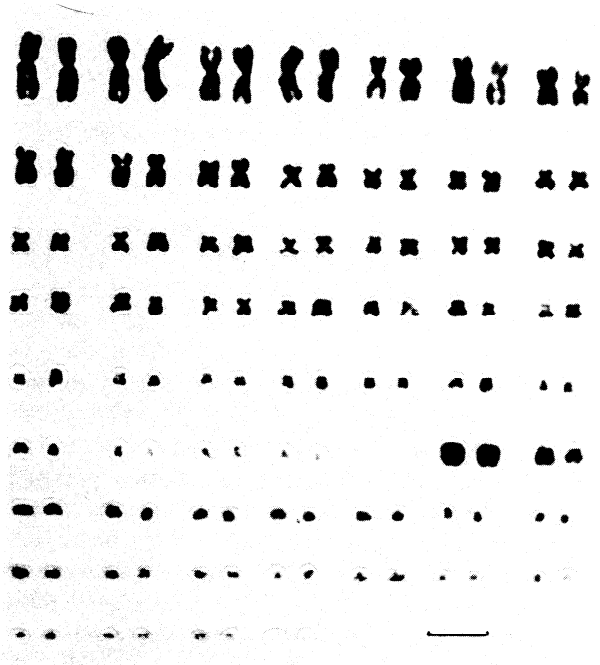

Fig. 1. Karyotype of *Acipenser ruthenus.* Bar, 5 μm.

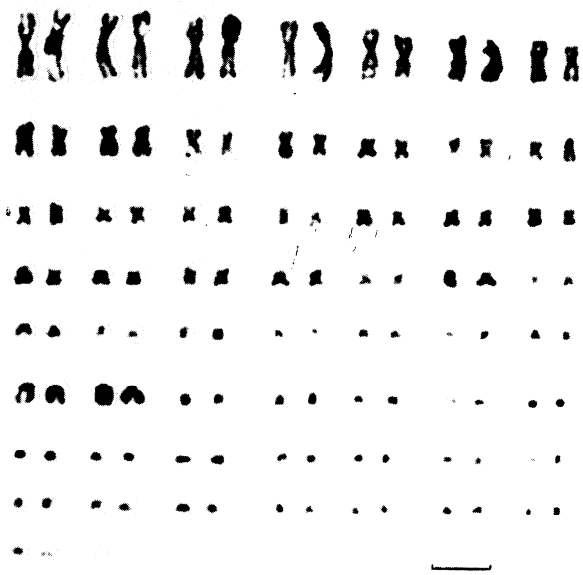

Fig. 2. Karyotype of *Acipenser stellatus.* Bar, 5 μm.

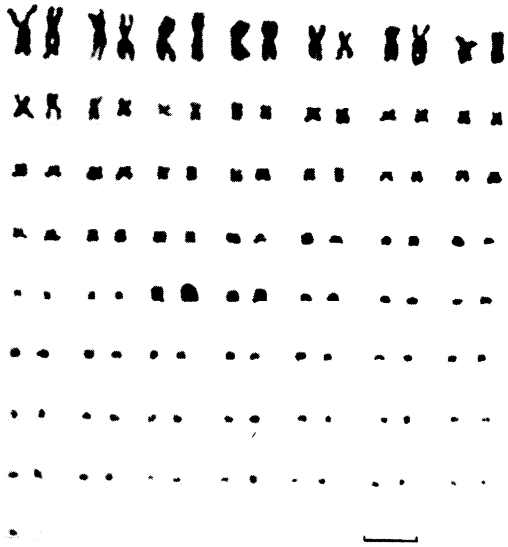

Fig. 3. Karyotype of *Huso huso.* Bar, 5 μm.

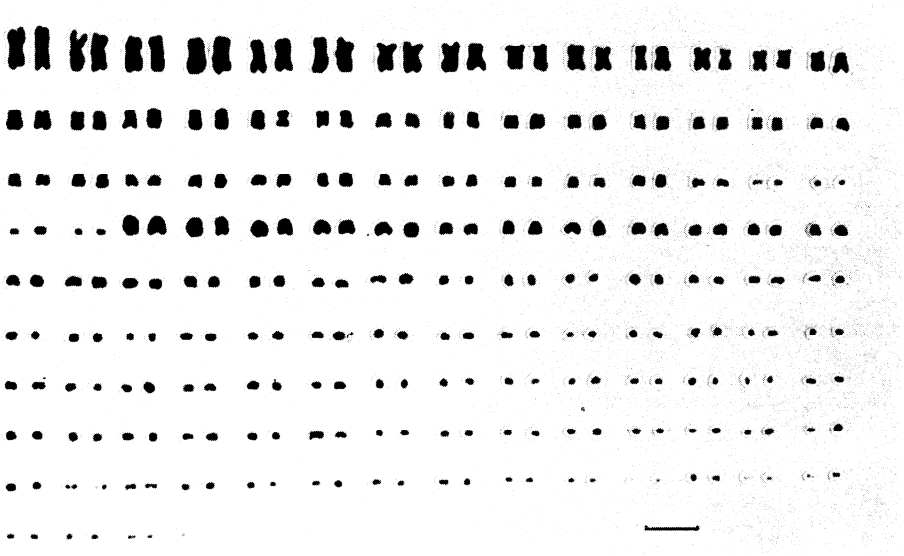

Fig. 4. Karyotype of *Acipenser gueldenstaedti.* Bar, 5 μm.

cies) of all nuclei, and the modal number of nucleoli in *A. stellatus* is 2–3, in *A. ruthenus* 2 (nuclei with 3 nucleoli are more rare), in *Huso huso* the number of nuclei having 3 nucleoli is 1.5 times less than those with 2 nucleoli. In all three species the number of nuclei with 4 and 5 nucleoli is approximately the same (11.8–15.0% and 5.3–6.7%

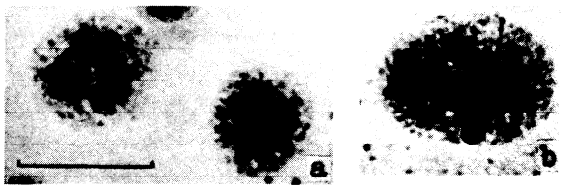

Fig. 5. Silver-stained nuclei of *A. stellatus* (a) and *A. gueldenstaedti* (b). Bar, 10 μm.

Table 1. Characteristics of karyotypes of four Acipenserid species.

Species	Number of fishes	Number of metaphases	2n	m + sm[a]	NF[b]
Acipenser gueldenstaedti	12	44	250 ± 8	92 ± 4	(250 ± 8) + (92 ± 4)
A. ruthenus	10	27	118 ± 2	82 ± 4	(118 ± 2) + (82 ± 4)
A. stellatus	9	26	118 ± 2	70 ± 4	(118 ± 2) + (70 ± 4)
Huso huso	12	42	118 ± 2	60 ± 2	(118 ± 2) + (60 ± 2)

[a] Number of meta- and submetacentric chromosomes.
[b] Number of arms.

Table 2. Number of nucleoli in Acipenserids (%)*.

Species	Nucleoli per nucleus											
	1	2	3	4	5	6	7	8	9	10	11	12
Acipenser gueldenstaedti	–	0.3	2.0	6.0	12.2	19.5	21.5	17.5	8.8	7.0	3.5	1.7
A. ruthenus	9.5	35.7	35.7	11.8	5.3	2.0	–	–	–	–	–	–
A. stellatus	10.3	36.7	32.0	13.0	6.5	1.5	–	–	–	–	–	–
Huso huso	15.0	37.8	25.5	15.0	5.7	1.0	–	–	–	–	–	–

* For each species 400 nuclei were analysed (100 nuclei on one slide of each individual).

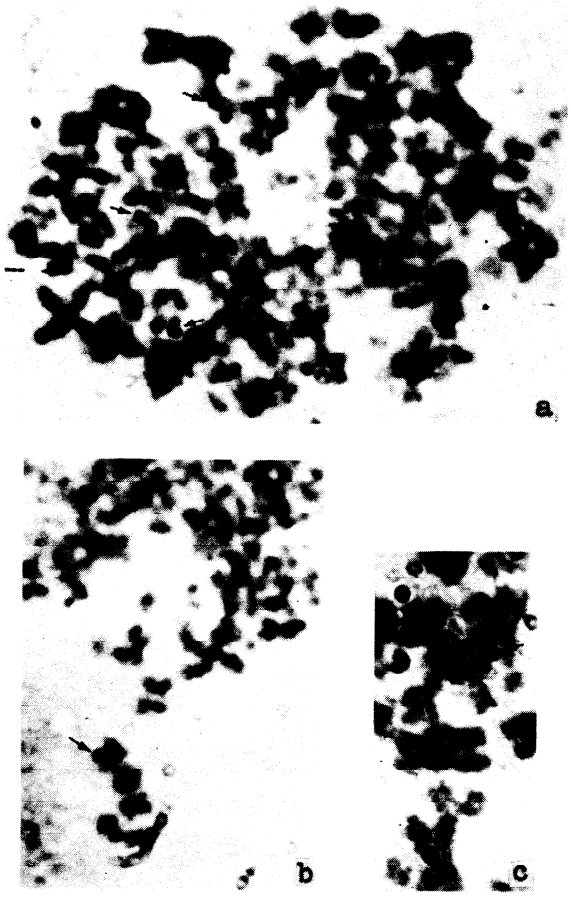

Fig. 6. Ag-AS-stained metaphase chromosome spreads of *A. ruthenus* (a), *A. stellatus* (b) and *H. huso* (c). The NORs are marked by arrows.

respectively), and only 1.0–2.0% of all nuclei are those having the maximum number (6) of nucleoli. The size of *A. gueldenstaedti* nuclei with a greater number of nucleoli is greater than that in the other three species.

It was impossible to localize the NORs in *A. gueldenstaedti* because of the high chromosome number (250±8) and superimposed position of many chromosomes on the slides. In *A. ruthenus* NORs are in the telomeric region of one pair of microchromosomes, and on at least one homologue of a pair of middle-sized acrocentrics (Fig. 6a). In *A. stellatus* and *H. huso* NORs are also located in the telomeric region of at least one pair of acrocentric microchromosomes (Fig. 6b, c).

Discussion

What is peculiar about the karyotypes of the four Acipenseridae species described is the high number of chromosomes (*A. stellatus, A. ruthenus, H. huso* 2n = 118±2, *A. gueldenstaedti* 2n = 250±8), and the high number of very small microchromosomes (about one third of the set); this coincides with the karyological data on other Acipenseriformes (Fontana & Colombo, 1974; Fontana *et al.,* 1977; Dingercus & Howell, 1976; Vasiliev *et al.,* 1980; Arefiev, 1983). Unlike our data, Fontana *et al.* (Fontana & Colombo, 1974; Fontana *et al.,* 1977) cite different chromosome numbers of Western European Acipenseridae; according to their results, *H. huso* has 116±4 (68 meta- and submeta-) chromosomes, and *A. ruthenus* 116±4 (72 meta- and submeta-) chromosomes. It seems possible that the difference between our and Fontana's data is a difference in the classification of the smallest chromosomes.

There are different numbers of active nucleoli in the nuclei of the four species investigated: 2–3 in *A. stellatus* and *A. ruthenus,* 2 (and more rarely 3) in *H. huso* (the maximum number of nucleoli is 6 in all three species), and 6–8 (maximum 11–12) in *A. gueldenstaedti.* As had been found earlier, the nuclei of *Polyodon spathula* (family Polyodontidae of the same order Acipenseridae) have 4 (the mean number) active Ag-staining nucleoli (Dingercus & Howell, 1976).

Unusually high diploid numbers as well as the numbers of NORs and nucleoli indicate the polyploid origin of Acipenseriformes. The polyploid origin characterizes not only the species with the large set, 2n = 240–260 (*A. gueldenstaedti, A. baeri, A. naccarii*), but also those with a 120-chromosome set. Dingercus and Howell (1976) divided the karyotype of *Polyodon spathula* into groups consisting of four chromosomes of similar morphology. Moreover, they found 4 active nucleoli per nucleus in this species. They concluded that *P. spathula* is a tetraploid. In spite of some difficulties, one can agree with the division of the *P. spathula* karyotype into 30 groups of 4 chromosomes each; the same procedure (with greater uncertainty) can be applied in the case of *A. ruthenus* or *H. huso* karyotypes.

Some authors consider that the tetraploidization of 60-chromosome ancestors of contemporary

Acipenseriformes occurred about 300 Myr ago, before the radiation of this order (Dingercus & Howell, 1976; Carlson *et al.,* 1982). According to paleontological data, the age of the order Acipenseriformes is only about 200 Myr (Berg, 1955), and the age of the family Acipenseridae, 80 Myr. The diploid numbers of Holostei (the closest relatives of Acipenseriformes) are also about 60: *Lepisosteus oculatus* has 2n = 68 (Ohno *et al.,* 1969b), *L. osseus* has 2n = 56 (Ojima & Yamano, 1980b). But many karyological changes seem to have occurred in the course of the evolution of the order Lepidosteiformes: the karyotype of *L. oculatus* consists of many meta- and acrocentric macrochromosomes, as well as of many microchromosomes, but in the *L. osseus* karyotype microchromosomes are absent. The karyotype of *Amia calva* (order Amiiformes, closely related to Lepidosteiformes), is more reduced, 2n = 46, but it still includes microchromosomes (Ohno *et al.,* 1969b).

The similarity between karyotypes of species with a 120-chromosome set points to the low rate of their karyological evolution. This correlates with the low rate of DNA evolution: practically all genome fractions, the repeated and unique sequences, in *A. ruthenus, A. stellatus, A. gueldenstaedti* and *H. huso* DNA are homologous, the number of nucleotide substitutions in the first fraction is 0–2.6%, and 1.5–2.7% in the second (Kedrova *et al.,* 1980). Besides, a low degree of protein evolution is a characteristic feature of the American Acipenseriformes. These have a very low degree of protein polymorphism: for *P. spathula* (2n = 120), *Scaphirhynchus platorhynchus* (2n = 112±, Ohno *et al.,* 1969b) and *S. albus* $\bar{H}$ (mean heterozygosity) is between 0.010 and 0.017 (Carlson *et al.,* 1982; Phelps & Allendorf, 1983), whereas for the other Osteichthyes mean heterozygosity is 0.0513 (Nevo, 1978).

As the species with large sets – *A. gueldenstaedti* (this paper), *A. baeri* (Vasiliev *et al.,* 1980) and *A. naccarii* (Fontana & Colombo, 1974; Fontana, 1976) – are closely related taxonomically, they may be the descendants of one tetraploid (octoploid) ancestor form, or their origin may be a result of parallel and independent instances of tetraploidization. Two-fold increase in chromosome number correlates with genome increasing: in *A. sturio* and *H. huso,* 2n = 120, the DNA content reaches 3.6 pg per nucleus, in *A. naccarii,* 2n = 240, 2C = 5.7–6.3 pg per nucleus (Fontana, 1976). Since the lowest number of nucleotide substitutions was found in *A. gueldenstaedti* and *A. ruthenus* DNA (in comparison to *A. stellatus* and *H. huso* DNA – Kedrova *et al.,* 1980), one can conclude that *A. gueldenstaedti* originated from (an) ancestral form(s) related to *A. ruthenus.*

Two genetical pecularities, polyploidy and the existence of a great number of microchromosomes in the karyotype, favoured, apparently, the Acipenseriformes to become 'living fossils'. The number of functional nucleoli per nucleus in somatic cells may be considered as an illustration of independent and repeated cases of polyploidization in the course of acipenseriform evolution. Data on the number of nucleoli, NORs and their position in the karyotypes of different fishes are summarized in Table 3. The overwhelming majority of species have two nucleoli per nucleus and two NORs per karyotype, the latter usually located in telomeric regions of chromosomes. The exceptions are the diploid species *Gobius fallax* (Gobiidae), a few Cyprinidae diploid species, and especially polyploid species of the families Salmonidae and Cyprinidae as well as Acipenseriformes.

Now it is generally accepted that the family Salmonidae consists of tetraploid species (in respect to Clupeidae and some other Salmonoidei); probably the tetraploidization occurred about 40–50 Myr ago (Ohno *et al.,* 1969a; cf. review by Allendorf & Thorgaard, 1984). The set of the supposed tetraploid ancestor must (have) consist(ed) of 96–100 chromosomes (Simon, 1963; Ohno *et al.,* 1969a; Vasiliev, 1985); it underwent diploidization, which seems to be going on even today. Centric fusions prevailed in the course of chromosomal diploidization; at the same time, functional diploidization took place. The latter resulted in the loss of expression ('silencing') of about half of all genes (Allendorf *et al.,* 1975; Vuorinen, 1984). In salmonids which underwent substantial chromosomal diploidization (2n = 52–68), two NORs per set are active (as in diploid species), while in salmonids with 78–84 chromosomes 4 and even 10 NORs per set are functional (Phillips, 1983; Table 3).

As many other cyprinids, *Carassius auratus* is of a tetraploid origin (Ohno *et al.,* 1967); in this species the tetraploidization seems to have occurred

Table 3. Numbers of nucleoli (N) and nucleolus organizer regions (NOR) and NOR location in fish karyotypes (AG-AS-staining data).

Species	2n	N number per nucleus	NOR number per karyotype	Chromosome, location[a]	References
Chondrichthyes					
Elasmobranchii					
Scylliorhinidae					
Scylliorhinus canicula	62	2	2	sm, 20p (large microchromosomes)	Schmid *et al.*, 1982
Osteichthyes					
Crossopterigii					
Coelacanthiformes					
Latimeria chalumnae	–	2	–	–	Dingercus, 1979
Dipnoi					
Lepidosirenidae					
Lepidosiren paradoxa	38	2[b]	–	–	Dingercus, 1979
Actinopterygii					
Acipenseriformes					
Acipenseridae					
Huso huso	118 ± 2	2 (3)	2 and more	at least 2 pairs of microchromosomes, t	This paper
Acipenser ruthenus	118 ± 2	2 – 3	2 and more	a (middle-sized) and a pair of microchromosomes, t	This paper
A. stellatus	118 ± 2	2 – 3	2 and more	microchromosomes, t	This paper
A. gueldenstaedti	250 ± 8	6 – 8	–	–	This paper
Polyodontidae					
Polyodon spathula	120	4	–	–	Dingercus & Howell, 1976
Teleostei					
Salmonidae					
Salmo trutta	80	–	4	a (small-sized, p)	Phillips, 1983
S. gairdneri	58 – 62	–	2	m, 22p	Schmid *et al.*, 1982; Phillips, 1983
Salvelinus alpinus	78 – 82	–	4	two pairs, m & a	Phillips, 1983
S. namaycush	82	–	10	m (one pair, t); a (two pairs, t)	Phillips, 1983
S. fontinalis	84	–	10	m (one pair, t); a (two pairs, t)	Phillips, 1983
Oncorhynchus gorbusha	52 – 54	–	2	m	Phillips, 1983
O. nerka	56 – 58	–	2	m	Phillips, 1983
O. kisutch	58	–	2	a	Phillips, 1983
O. masu	66	–	2	a	Phillips, 1983
O. tschawytscha	68	–	2	a	Phillips, 1983
Umbridae					
Umbra limi	22	–	2	sm, 4q (t)	Kligerman & Bloom, 1977
Anguillidae					
Anguilla anguilla	38	–	2	sm, 8p or 11p	Wiberg, 1983; Sola *et al.*, 1983
A. rostrata	38	–	2	sm, 8p or 11p	Sola *et al.*, 1983
Ophichthyidae					
Ophisurus serpens	38	–	2	sm, 8p, t	Thode *et al.*, 1985
Serrasalmidae					
Serrasalmus spilopleura	60	–	5 – 10	a (five pairs)	Galetti *et al.*, 1985
Anostomidae					
Leporellus vittatus	54	–	2	m, 6q (t)	Galetti *et al.*, 1984
Leporinus elongatus	54	–	2	sm, 5q (t)	Galetti *et al.*, 1984
L. friderici	54	–	2	sm, 2q (t)	Galetti *et al.*, 1984
L. lacustris	54	–	2	sm, 2q (t)	Galetti *et al.*, 1984
L. obtusidens	54	–	2	m, 1p (t)	Galetti *et al.*, 1984
L. octofasciatus	54	–	2	sm, 2q	Galetti *et al.*, 1984
L. striatus	54	–	2	m, 1q (t)	Galetti *et al.*, 1984
Schizodon nasutus	54	–	2	m, 12q (t)	Galetti *et al.*, 1984
Parodontidae					
Apareiodon affinis	54 – 55	–	2	st (a pair of large chromosomes, q, t)	Moreira-Filho *et al.*, 1984
A. ibitiensis	54	–	2	st (a pair of large chromosomes, q, t)	Moreira-Filho *et al.*, 1984
A. pirracicabae	54	–	2	st (a pair of large chromosomes, q, t)	Moreira-Filho *et al.*, 1984
Parodon tortuosus	54	–	2	st (a pair of large chromosomes, q, t)	Moreira-Filho *et al.*, 1984

Table 3. Continued.

Species	2n	N number per nucleus	NOR number per karyotype	Chromosome, location[a]	References
Apteronotidae					
Apteronotus albifrons	24	-	2	sm, 4q	Almeida Toledo *et al.*, 1981; Foresti *et al.*, 1981
Eigenmannia sp.	29 – 31	2	2	a (c) or a & sm	Foresti *et al.*, 1981
E. virescens	38	-	2	sm (large)	Foresti *et al.*, 1981
Gymnotus carapo	52	-	2	sm (large, p)	Foresti *et al.*, 1981
Sternopygus macrurus	46	-	2	sm (large, q)	Foresti *et al.*, 1981
Cyprinidae					
Carassius auratus auratus	100	1 – 3	2 – 3	sm, 12p	Ojima & Yamano, 1981; Schmid *et al.*, 1982; Mayr *et al.*, 1985
C. a. buergeri	100	-	2	sm, 12p	Ojima & Yamano, 1981
C. a. cuvieri	100	-	2	sm, 12p	Ojima & Yamano, 1981
C. a. gibelio	160	1 – 4	4	sm	Mayr *et al.*, 1986
C. a. grandoculus	100	-	2	sm, 12p	Ojima & Yamano, 1981
C. a. langsdorfii	156	-	8	sm (middle-sized, p), three pairs of small-sized chromosomes	Ojima & Yamano, 1981
C. carassius	100	1 – 4	3	sm	Mayr *et al.*, 1986
Cyprinus carpio	100	1 – 2	2	sm	Wang *et al.*, 1985; Mayr *et al.*, 1986
C. c. chilia	100	-	2	sm	Wang *et al.*, 1985
C. longipectoralis	100	-	2	sm	Wang *et al.*, 1985
Gnathopogon elongatus	50	-	2	st (middle-sized, p, t)	Takai & Ojima, 1984
Ischikania steenackeri	48	-	2	st (middle-sized, p, t)	Takai & Ojima, 1984
Leucaspius delineatus	50	1 – 2	2	a, 24	Mayr *et al.*, 1986
Moroco jouyi	50	-	4	st (two pairs of middle-size, p, t)	Takai & Ojima, 1984
Notemigonus cryscencus	78	-	6	st (one pair of middle, two pairs of small size, p, t)	Takai & Ojima, 1984
Opsariichthys uncirostris	50	-	2	a	Gold & Ellinson, 1983
Pseudogobio esocinus	50	-	2	sm (middle-sized, p, t)	Takai & Ojima, 1984
Puntius (Barbus) tetrazone	50	2	-	-	Dingercus, 1979
Rhodeus ocellatus	48	-	2	st (small-sized, p, t)	Takai & Ojima, 1984
Scardinius erythrophthalmus	50	1 – 2	2	a, 23	Mayr *et al.*, 1986
Tinca tinca	50	1 – 2	2	m, 3p	Mayr *et al.*, 1986
Tribolodon hakonensis	50	-	4	st (two pairs of small size, p, t)	Takai & Ojima, 1984
Zacco platypus	50	-	4	st (large-sized) & sm (small-sized, p, t)	Takai & Ojima, 1984
Z. temminckii	50	-	8	st (large-sized), st (middle-sized), st (two pairs of small size), p, t	Takai & Ojima, 1984
Cyprinodontidae					
Fundulus diaphanus	-	-	2	sm, Xp & Yp (t)	Howell & Black, 1979
Oryziatidae					
Oryzias celebensis	36	-	2	sm (small), t	Uwa *et al.*, 1981
O. curvinotus	48	-	2	a (middle-sized)	Uwa *et al.*, 1982
O. javanicus	48	-	2	a (middle-sized)	Uwa & Iwata, 1981
O. latipes	48	-	2	sm, 6p (t)	Uwa & Ojima, 1981
O. melastigma	48	-	2	a (no. 1), c	Uwa *et al.*, 1983
Centrarchidae					
Pemoxis annularis	46	-	2	st	Gold & Ellinson, 1983
Percidae					
Perca fluviatilis	48	1 – 2	2	st, 16p	Mayr *et al.*, 1985
Cichlidae					
Saroterodon galileus	44	-	2	sm	Kornfield *et al.*, 1979
Tilapia rendalli	44	-	2	sm (t)	Kornfield *et al.*, 1979
Gobius fallax	42	2 (1 – 4)	4	a (4c, 14c)	Thode *et al.*, 1983

[a] m: meta-, sm: submeta-, a: acro-, st: subtelocentric chromosomes; q: long arm; p: short arm; t: telomeric; c: pericentromeric position.

[b] N are very large.

earlier than in salmonids. The diploidization took place only at the functional level (Leipoldt & Schmidtke, 1982; Leipoldt, 1983), since the chromosome number remained 100 (diploid cyprinids usually have 48–50 chromosomes; see reviews of Vasiliev, 1980, 1985; Arai, 1982). In accordance with the functional diploidization (which is very high, up to 80% of duplicated genes have been silenced – Woods & Buth, 1984), the individuals belonging to subspecies of *C. auratus* (which some authors consider as independent species), 2n = 100, have two active NORs per chromosome set, and in nuclei of triploid (hexaploid) *C. a. gibelio* and *C. a. langsdorfii,* 3n = 156–165, the number of nucleoli is two–four times greater (Ojima & Yamano, 1980; Mayr *et al.,* 1986; Table 3).

All these data make it possible to conclude that three of the acipenserids investigated (*A. ruthenus, A. stellatus* and especially *H. huso*) underwent intensive, but not complete, diploidization in terms of the number of active rDNA sites; *A. gueldenstaedti* is probably in the process of diploidization, as it has 'an excess' of active nucleoli. In *P. spathula* functional diploidization of rDNA did not occur, although at the level of structural genes it was finished. This species has only 6% of genes expressed as tetraploid loci (Carlson *et al.,* 1982); this level corresponds to the highest level of duplicated loci in diploid Osteichthyes. It seems possible that the control of the number of active rDNA sites is broken during tetraploidization; partly this change is connected with the change in parameters of tetraploid nuclei in comparison to diploid ones, and with the change in the rate of protein synthesis, etc. Subsequent diploidization of tetraploid genomes may occur at different rates of molecular evolution in di- and tetraploid lines originating from a common diploid ancestor form. In any case, the rate of fixation of base substitutions in rDNA genes of the tetraploid anuran *Hyla versicolor,* 4n = 48, has been considerably higher than the same rate in diploid *H. chrysoscelis,* 2n = 24, since their divergence (Toivonen *et al.,* 1983).

A high number of nucleoli points to real polyploidization, i.e. to the increase of the number of NORs correlated with the increase of the chromosome number. If genome size increases by endoreplication of DNA without change of chromosome number, the number of nucleoli remains 2, but their size increases considerably. Dipnoans with their great genomes may illustrate this situation: there are two Ag-staining nucleoli of big size in very large nuclei (Dingercus, 1979) of *Lepidosiren paradoxa,* 2n = 38 (2C = 241, 4 pg, Pedersen, 1971).

The maximum number of nucleoli per nucleus in acipenserids is very unusual: tetraploids have 6, *A. gueldenstaedti* has 12 nucleoli instead of 4, or 8 if one assumes two nucleoli to be normal for diploids. In other words, in all four species of acipenserids NORs must be located on three chromosome pairs. In *A. ruthenus* NORs are on at least two pairs of chromosomes – on a pair of acrocentric microchromosomes and on a pair of middle-size acrocentrics. Localization of NORs on more than one chromosome pair is found almost exclusively in tetraploid forms of fishes (and anurans): salmonids, *C. a. langsdorfii,* 3n, and tetraploid *Odontophrynus americanus,* 4n = 44, Anura (Ruiz *et al.,* 1981). Among urodelans only in *Triturus vulgaris meridionalis* are rDNA sites located outside NORs (Nardi *et al.,* 1978). Many fishes may have 'additional' sites of rDNA, which are inactive in somatic cells: for instance, in spermatozoids of *Tilapia rendalli* two sites of rDNA, which are inactive in somatic tissues, are activated (Foresti *et al.,* 1983). One can explain the situation in another way: in tetraploid fishes and amphibians a part of the rRNA genes may be translocated from NORs to other sites of the genome. For such transposition in plants it was proposed that rRNA genes can be mobile elements (reviews of Vasiliev, 1980, 1985; Arai, 1982; Schubert, 1984).

All data discussed confirm our conclusion on the polyploid (tetra- and octoploid) origin of contemporary Acipenseriformes. It seems possible that all four species investigated have a mechanism to compensate the number of active nucleoli, which controls the functional diploidization of the whole genome in these polyploids.

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